(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 705 842 A2

(12) **EUROPEAN PATENT APPLICATION**

(43) Date of publication:
**10.04.1996 Bulletin 1996/15**

(51) Int. Cl.[6]: **C07K 14/00**, C12Q 1/68

(21) Application number: **95115510.0**

(22) Date of filing: **02.10.1995**

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE**

(30) Priority: **06.10.1994 EP 94115751**

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(54) **Regulated genes by stimulation of chondrocytes with 1L-1beta**

(57) The present invention refers to the novel use of osteopontin, calnexin and TSG-6 gene product in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1β and their use in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues.

EP 0 705 842 A2



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## Description

The present invention refers to the novel use of osteopontin and calnexin in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1β and their use in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues.

Among the diverse biological effect of interleukin-1 (IL-1), are its actions on the metabolism of many connective tissue cell types including articular chondrocytes. IL-1 inhibits proteoglycan (PG) synthesis by chondrocytes and stimulates production of prostaglandin $E_2$ and metallo-proteinases capable of degrading matrix macromolecules. From experimental results, and from findings of IL-1, PG fragments and proteolytic enzymes in inflamed joints, it was deduced that IL-1 plays a role in cartilage degradation in osteoarthritis and rheumatoid arthritis (Benton HP & Tyler JA. 1988, Biochem, Biophys. Res. Comm. 154, 421-428; Aydelotte MB et al. Conn. Tiss. Res. 28, 143-159; Wood DD et at., Arthrithis Rheum. 26, 975-983; Lohmander LS et al., Trans Orthrop. Res. Soc. 17, 273). Matrix metalloproteinases are potential candidates for drug interaction at the enzyme level, but relevant molecular targets interfering with earlier processes leading to cartilage degradation are still lacking. Therefore, one objective of the present invention was to identify potential targets for drug modification of IL-1β induced cartilage degradation on the RNA level of human articular chondrocytes from osteoarthritic cartilage.

As an initial attempt to investigate differentially expressed genes in diseased cartilage, total RNA from IL-1β stimulated and unstimulated human chondrocytes was subjected to differential display of mRNA by reverse transcription and polymerase chain reaction (DDRT-PCR). This method can be used to identify and isolate those genes that are differentially expressed in two cell populations (Liang P & Pardee AB 1992, Science 257, 967-971; Liang P et al., AB 1993, Nucl. Acids Res. 21, 3269-3275; Bauer D et al. 1993, Nucl. Acids Res. 21, 4272-4280). The key element is to use a set of oligonucleotide primers, one hybridizing to the polyadenylated tail of mRNAs, the other being arbitrary decamers that anneal at different positions relative to the first primer. mRNA subpopulations defined by these primer pairs are amplified after reverse transcription and resolved on DNA sequencing gels. Band patterns are created, which are characteristic for each RNA population extracted from the cell population under study. For example, 100 different primer combinations should generate a total of approximately 10,000 PCR products for each population, which should represent about the half of all expressed cellular genes. A comparison of the band pattern obtained from two cell populations reveals differentially displayed bands which correspond to differentially expressed genes. Subsequently, differentially displayed bands can be extracted from the gel, reamplified, subcloned and sequenced.

Due to its extreme sensitivity, the appearance of artifactual bands is an inherent problem of the DDRT-PCR method used according to the present application. An additional problem is also the evaluation of complex gene expression patterns. Yet another problem of the present invention is that only minute amounts of RNA are available.

Therefore, it was particularly surprising that the DNA TAU1/1 with the sequences

```
TAU1/1(1)
ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC ATCCCCGTTT    60
CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG GAAGGACAGT TATGAAACGA   120
GTCAGCTGGA TGACCAGAGT GCTGAAACCC ACAGCCACAA GCAGTCCAGA TTATATAAGC   180
GGAAA                                                               185
```

and

```
TAU1/1(2)
CTAAATGCAA AGTGAGAAAT TGTATTTTTT CTCCTTTTAA TTGACCTCAG AAGATGCACT    60
ATCTAATTCA TGAGAAATAC GAAATTTCAG GTGTTTATCT TCTTCCTTAC TTTTGGGG     118
```

and the DNA TTU2/2 with the sequence

```
AACCAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT TGTTTTCTTA    60
TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG CATGTTTTTC TTCATAGATA   120
CCCGTTTTGG GGTTTTTGCG TCGGAAGATG AATGGCATTT ATAGTCCTCT CCACATTTAT   180
CTG                                                                 183
```

are 100 % identical to human osteopontin cDNA and 97.2 identical to human calnexin, respectively. This demonstrates that the experimental approach of the present invention worked efficiently, i.e. the use of 100 different primer combinations (25 oligodecamer primers, $4T_{12}$MN-primers) generated a total of approximately 10.000 PCR products for each population which represent 53 % of all expressed cellular genes. 123 PCR bands out of 10.000 appeared as differentially expressed bands. 53 of the original 123 PCR bands were reproducibly displayed by comparing the PCR band patterns from two patients; of those 68 % arose from IL-1β stimulated chondrocytes.

It was further found that osteopontin which is a secreted highly acidic phosphoprotein of 32 kd (Denhardt and Guo (1993) FASEB J. 7, 1475-1482) is surprisingly downregulated in IL-1β stimulated human chondrocytes. This means that osteopontin is involved in IL-1β related diseases of connective tissues, in particular osteoarthritis.

Osteoarthritis is characterized as a slowly progressing matrix degeneration with continuing degradation of collagens and proteoglycans and subsequent release of matrix fragments into the synovial fluid. Any disturbance of the normal chondrocyte matrix interactions, for example through a loss of osteopontin, could cause an altered signaling through the integrin $alpha_v beta_1$ and thus changed cellular responses leading to early steps of matrix degradation.

Therefore, one embodiment of the present invention is the use of osteopontin itself or parts thereof, antibodies against it or nucleic acids such as DNA or RNA or parts thereof coding for osteopontin or parts thereof in the diagnoses, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. According to the present application the term "parts" means either at least 8, preferably 12, in particular 15 amino acids in case of proteins or 6-100, preferably 10-40, in particular 12-25 nucleic acids in case of DNA or RNA as hybridization probes. The methods of diagnosing such diseases will be described infra. In addition, quantification on the protein level is possible with osteopontin specific antibodies on Western blots, in immunochemistry, FACS analysis or ELISA based assay systems. The present invention refers also to a diagnosis aid or a pharmaceutical for such use. Osteopontin can be produced for example recombinantly through expression in procayotes, in insect cells in mammalian cells or in mammalian cells using Vaccinia as detailed in Ausubel et al. 1994 [Current protocols in molecular biology, Chapter 16, John Wiley & Sons, Inc]. The cDNA of Osteopontin is e.g. disclosed in Young et al. (1990), Genomics 7, 491 - 502.

Antibodies against osteopontin can be generally produced for example by the method of Neil GA & Urnovitz HB (Trends in Biotechnology, 6, 209-213, 1988) or Köhler G & Milstein C (Nature, 256, 52-53, 1975).

Also calnexin which is an integral membrane protein of 88 kd (Bergeron et al. (1994) TIBS 19, 124-128) is surprisingly downregulated in IL-1β stimulated human chondrocytes compared to unstimulated chondrocytes. This means also that calnexin is involved in IL-1β related diseases of connective tissues, in particular osteoarthritis. In addition, a downregulation of the calnexin synthesis would cause a reduced amount of correctly and completely folded proteoglycans because calnexin is a new type of molecular chaperone that associates with incompletely folded proteins such as proteoglycans. Proteoglycans are highly glycosylated glycoproteins which are of central importance for the maintenance of the cartilage tissue integrity.

Hence, an additional embodiment of the present invention is the use of calnexin itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

Calnexin can be produced for example recombinantly as described above for osteopontin. The cDNA of Calnexin is e.g. disclosed in Galvin et al. (1992), Proc. Natl. Acad. Sci. USA 89, 8452 - 8456. The production of said antibodies are also generally described above.

Potential role of identified cDNA fragments in IL-1 mediated cellular processes TSG-6

A homology search in the GenBank and EMBL databases revealed a 99.5 % sequence indentity of fragment TAU7/2(c) with the gene coding for human TSG-6. TSG-6 (TNF stimulated gene 6) was originally isolated by differential cDNA library screening as a TNF induced gene sequence from human fibroblasts (Lee et al., 1990). It was further characterized by Lee et al (1992) as a TNF and IL-1 inducible, secretory, 39 kDa glycoprotein with extensive sequence homology with a region implicated in hyaluronate binding, present in cartilage link protein, proteoglycan core proteins,

and the adhesion receptor CD44. With the ability to bind HA and with the most extensive sequence homology to CD44, TSG-6 belongs to the hyaladherin family. Wisniewski et al. (1993) detected high levels of TSG-6 protein in synovial fluids of patients with various forms of arthritis. Six normal control patients did not contain detectable TSG-6 protein in their joint fluid, whereas joint fluids from nine rheumatoid arthritis patients contained high, moderate or low levels of TSG-6. Two patients with osteoarthritis had high levels of TSG-6 in their joint fluids. The apparent local source of TSG-6 in the joints are synoviocytes and chondrocytes (Wisniewski et al., 1993). Lee et al. (1992) speculated that TSG-6 could act as a competitive inhibitor of the interaction between CD44 and its ligand(s) and thus might influence the structural organization of the extracellular matrix of connective tissue, resulting in a destabilization of the proteoglycan aggregates.

Hence, an additional embodiment of the present invention is the use of TSG-6 gene product itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

Fibronectin

A homology search in the GenBank and EMBL databases revealed a 100 % sequence identity of fragment TTO20/1(c) with the gene coding for human fibronectin.

Fibronectin is a 450 kd glycoprotein with various functions. It acts as an adhesive ligand, as growth or differentiation factor and has chemotactic properties. It is found in the extracellular matrix of most types of cells (Hynes R 1993. Fibronectins, In: Guidebook to the extracellular matrix and adhesion proteins. Editors: Kreis T, Vale R. Oxford University Press. 56-58). An enhanced accumulation of fibronectin and fragments derived from it are found in the synovial fluid and on the inflamed synovial and pannus surfaces in the knee joint of patients with rheumatoid arthritis (Dutu A, Vlaicu-Rus V, Bolosiu HD, Parasca I, Cristea A. 1986. Fibronectin in plasma and synovial fluid of patients with rheumatic diseases. Med. Interne 24, 61-68). Patients with osteoarthritis, as well, have greatly increased levels of fibronectin in their synovial fluid and on cartilage surfaces (Xie D-L, Meyers R, Homandberg GA. 1992. Fibronectin fragments in osteoarthritic synovial fluid. J. Rheumatology 19, 1448-1452). The intraarticular injection of fibronectin fragments causes a severe depletion of cartilage proteoglycans in vivo (Homandberg GA, Meyers R, Williams JM. 1993. Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans in vivo. J. of Rheumatology 20, 1378-1382), which is explained by the induced relase of several proteinases, including stromelysin (Xie D-L, Hui F, Meyers R, Homandberg GA. 1994. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: Stromelysin plays a major role in chondrolysis. Arch. Biochem. and Biophysics 311, 205-212). At high concentrations, fibronectin fragments enhance cartilage catabolism through release of cytokines, including IL-1 (Homandberg et al., personal communication).

In respect to these published data, the upregulation of fibronectin by IL-1 can be regarded as a positive feedback regulation, enhancing the self destructive potential of chondrocytes and synoviocytes. With this, fibronectin expression is a direct pharmacological target.

In addition, the sequencing of differentially displayed PCR products discovered also unknown DNA fragments which correspond to differentially expressed genes with or without stimulation of chondrocytes with IL-1β.

Therefore, another embodiment of the present invention is a DNA containing a DNA selected from the group consisting of

TA08/2(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | CCAAGTTTTT | CCAGCAACCC | CAAGGGAATA | CAGGGAGATC | AATGCACCA |
| 51 | AAATGGGAAA | AGAAAAATAC | TTCGATGCAA | TGAAACAAAG | CCTTTTTCCG |
| 101 | TTCAGTTTCC | ATAATTCAGT | GGTCAGTTTT | AAGGCTGCCA | CTTGGG |

TA016/1(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | GACACGAACA | CCACATATTT | TTATTGGAGG | CCCCATGGCT | CCTTGGAAGC |
| 51 | CATTTTGGAA | CCAAGGGGAC | CCACCTTTTT | | |

TA016/2(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | CTAAATATAT | TCTCTAACAA | GTTAATCTCT | TTCAAATCTA | TAGATAAAAC |
| 51 | TAAAAGGATA | AGGAACCAAG | GTTTAACCGA | CCTAGCCAAT | TATGGCAATC |
| 101 | ATACTTGCTT | TTTAG | | | |

TA017(C)

```
  1  CATGAAATAT  TTCTTGAGGT  AATAAGCTTT  TACCAAGCTT  ATATTTTTGG
 51  GCAATTCAGT  TACAATGAGA  AAAAAACACA  CCAAAAGACC  AAAAATTTTA

101  AAAACTCACT  TTTCTTGCAA  TCATAGACAT  TTGCATTATT  ATAGAACATT
151  CAAACAAGTT  AGGTGGATAA  TTATTGTCTA  TAGATAAATA  CGATGCAATT
201  TTTTTAATGT  ATGACCGATA  CTCCGTATAT  ACTTAGATAA  CTTATCCAGA
301  AACCTCAACT  GTATTGAACA  TTGCTGAGAG  AAATCAACAA  TAATTTTAAC
351  AGATATGATG  ACAGNAAAAA  TTGATTGCAT  ATCTTTTTGC  ACTAAAACTT
401  TTATATTTAT  TT
```

TA019(C)

```
  1  AGAGCAGGGG  TATTTCNCGG  TTCATACCGC  CATGGCTTAA  GAAGCAAAAG
 51  TCATATACCT  TAGTAGTGGC  AAAGATNGAG  GAGATAAAAA  AGAGCCTACC
101  CAAGCTGTTG  TTGAAGAACA  GGTCTTAGAT  AAAGAGGAAC  CCTTCCAGAA
151  GNACAGAGAC  AGGCTAAGGG  TGATGCTGAG  GAAATGGCTC  AGAAGAAACA
201  AGAGATTAA
```

TAU 1/1(2)

```
  1  CTAAATGCAA  AGTGAGAAAT  TGTATTTTTT  CTCCTTTTAA  TTGACCTCAG
 51  AAGATGCACT  ATCTAATTCA  TGAGAAATAC  GAAATTTCAG  GTGTTTATCT
101  TCTTCCTTAC  TTTTGGGG
```

TAU 1/1(1)

```
  1  ACATCACCTC  ACACATGGAA  AGCGAGGAGT  TGAATGGTGC  ATACAAGGCC
 51  ATCCCCGTTT  CCCAGGACCT  GAACCCGCCT  TCTGATTGGG  ACAGCCGTGG
101  GAAGGACAGT  TATGAAACGA  GTCAGCTGGA  TGACCAGAGT  GCTGAAACCC
151  ACAGCCACAA  GCAGTCCAGA  TTATATAAGC  GGAAA
```

TAU1/2(2)

```
  1  CCGGAATGGG  GAGCAAACTA  TAAGAACCGG  GACCAGTTTC  CTCTCTTTGT
 51  GCCCTAGTTC  CCCCTCCTTT  GTATACACCC  TCCATCCTGA  ATAGACTCTG
101  GTTCTCAGCG  TAACACCGAC  AACATTCAAT  CCTGTAGAGA  AACAAATGTT
151  AGCTCAGAAG  GACACAGCCT  TTGAATCATC  AGAGAGTT
```

TAU 7/1(2)

```
  1  GTTAAGAATA  ACTAAATAAA  AGTTTTAATT  AATTTAGGAA  TATAAAAAAC
 51  TATTAACATT  TAATTTTATA  ACTGTATCTG  CCAAGCAACT  TTAAATATAA
101  TTTATTTACC
```

TAU 7/1(1)

```
  1  CACGCAATGT  GAAATAGGCA  CATAGGAAGA  ATGGGGAAAC  CATCCCCTCA
 51  AGCATTTATC  CTTTGAGTTA  CAAGCAATCC  AATTACACTC  TTTTAGTTAT
101  TTTTAAATGT  ACAGTTAGGT  TATTA
```

TAU 7/2(C)

```
  1  CCTTGAAGAT  GACCCAGGTT  NCTTGGCTGA  TTATGTTGAA  ATATAGACA
 51  GTTACGATGA  TGTCCATGGC  TTTGTGGGAA  GATACTGTGG  AGATGAGCTT
101  CCAGATGACA  TCATCAGTAC  AGGAAATGTC  ATGACCTTGA  AGTTTCTAAG
151  TGATGCTTCA  GTGACAGCTG  GAGGTTTCCA  AATCAAATAT  GTTGCAATGG
201  AT
```

TAU10(1)

```
  1  GGAGATGACA  TTTGCTTTGG  GCAGAGGCAG  CTAGCCAGGA  CACATTTCCA
 51  CTATAATTTT  ACAAAGTTAA  ATTTATAAGC  TAGCATTAAG  TAAAGTGAAG
101  TTCCAGCTCC  CTTGCTAAAA  ATAACTAGAG  GTAATAATTG  GTATTCAGGT
151  AACTCATTTA  CATCATAATG  TGTTGTGAAA  A
```

TAU12/1(2)

```
  1  TATAAAATAT  AAATTATATT  ATAAATCATG  TATTATTTAT  AAAATTATAT
 51  TATAAATTTA  TAAAAATATA  AATTATATTT  TAGGCTTAAT  GTATAAGGAA
101  TATAAATTAT  TAATAAGCAT  ATGA
```

TAU 12/1(1)

```
  1  TGTAATTAAC  TGTNCTTGTA  GGTCTGTCTT  TTATACATGT  GTGAGTTTTT
 51  CTTTACAATA  GATTCCTAGC  ATTGGGATTG  CTAGGTCAGA  TGGTATGCAC
101  ATTTGACATT  TTGATTGATA  GCACCAGATT  GCTTTGTTAA  AAAATTTTNN
151  TTTATAGTTT  ACATTATCTT  TGTACAATAG  ATGTTCTCTT  TCGAC
```

TAU 12/2(1)

```
  1  GGGAAGTGAA  TTGAAAATAC  TTCTTTNTCA  ACATAATTTT  NGGGTTTTGA
 51  AATTGTGTTT  GGGTTTTCAG  GAAATTGGTG  GTAATCTTGT  ATTAGACTGAA
101  AAAAAGTGAA  TTTTAAAATT  CTCAGTGAAG  AAGCAAATGA  TTTATTTTTC
151  ATAGA
```

TAU12/3(2)

```
  1  TGTTCTGGTA  ACTGTTCTAA  TTGTGTCTTT  GTTACTTCCA  GTGCAACCCT
 51  TTCAGGTAAG
```

TAU12/3(1)

```
  1  CTAAAGAACT  TGGTATCTCT  ATTAAAGCAC  ACGAACCTCC  AAGGAAAATA
 51  GAGCGATTTA  CTCTTCTCAT  ATCAGTGCAT  ATTTATAAGA  AGCACGGAGT
101  CA
```

TAU13/1(1)

```
  1  AGTCATCAAT  TCCTTTTTAT  CTGTAATTAC  ACATTTGTTT  TTATTTCAAA
 51  GTAATTATAA  GGTGTTATAT  TGCATATAAT  CAGAAAACTA  AATGGAAATA
101  AAATTTTAGT  AAGCCCGGCC  CCTTTGACCG  ATACAGAAAA  CTTGA
```

TAU 13/3(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | TATATGGCAG | TCTAAAGCAT | CAAAGATTTG | CATCAACATC | TTTCATTTTA |
| 51 | GACATCTCCT | TGCAATGTAA | AATATCATGT | ATCAACAACA | TCTGGTGCAA |
| 101 | ATCCATGAGT | CTAACTCGAC | ATTCATCTTA | GCTCGATTAT | TATTCCTTCG |
| 151 | TACAGTCGAT | GTAAACAATA | CAGAAAGAGG | ATTATTAAGA | ACAGTTT |

TAU 13/3(1)

| | | | | | |
|---|---|---|---|---|---|
| 1 | ATTCATGAAA | TGGTCTATAT | GCATGATATT | GTAAATTCGG | ACTCGAAACC |
| 51 | GAAACCAAGG | ATTCCGTTAC | AAAAATTCCT | TAATGCTGAG | AATGTTCTCA |
| 101 | CGCAAACAAC | ATCATGGACA | TTAAATTCAA | GATATGTGAA | TGTTAATTCT |
| 151 | GTCAATAAAG | TCAACGTAAA | GAGTAAAGTT | AAAAACAGTT | ATATCTNNNC |
| 201 | TGTCAATGAT | GAGTTTAGTT | TAACAGATGA | TGAATCAATT | CT |

TCO 16/1(C)

| | | | | | |
|---|---|---|---|---|---|
| 1 | CAAAGTGTTT | TTGGTTTTGA | GAGAGAGAGA | GATTGAGAGA | CAGAGAGAGA |
| 51 | GAGAGAAACC | AAGGGATCAT | GATAGTTATA | GTCAAATACG | AGGTTGGATT |
| 101 | ATCTTTTGAA | AATGTGTTGG | TTCTGTGATA | CAAGAGGAAG | CTAAGACATA |
| 151 | TCGTGGAAAC | ATCTCCCCCC | TCCACCTTAA | TATCAAGAAC | AAATTGTGGA |
| 201 | ATCTAATGTT | AATGAGAAGT | AGTTCCCCAC | TGTGTCAGAT | G |

TCO16/2(C)

| | | | | | |
|---|---|---|---|---|---|
| 1 | NCATCTGACA | CAGTGGGGAA | CTACTTCTCA | TTAACATTAG | ATTCCACAAT |
| 51 | TTNNNCTTGA | TATTAAGGNN | NNNNNGGGAG | ATCGTTTCAC | GATATCGTCT |
| 101 | TAGCTTCCTC | TTGTATCACA | GAACCAACAC | ATTTCAAAAG | ATAATCCTTC |
| 151 | CTCNNTTTGA | CTATAACTAT | CATGATCCCT | TGGTTCTCTC | TCTCTCTCTG |
| 201 | CTCTCTCATC | TCTCTCTCTC | TNAAAACNAA | | |

TCO17(C)

| | | | | | |
|---|---|---|---|---|---|
| 1 | ACAGTAGTTA | GGAGTTTCTT | TACTTACAAA | ATCACTGGAA | ATGATTAAAT |
| 51 | TGCTTTTCCC | CCTCCCCAGA | GGTGCATTTT | TCTTATTTCC | ATATAGTAAA |
| 101 | GTTGAGCTTT | TACAGTGCAT | AATGTGACAT | TTGGAATGCT | TATCAACTGC |
| 151 | ATGTAAACAT | TAATAACCT | | | |

TCO18(C)

| | | | | | |
|---|---|---|---|---|---|
| 1 | GTAAATGGTA | TTANÑNGCTG | AAGAAAAAAA | ATTTTTCAAG | ACCTCTGTTT |
| 51 | TTTAACTGAA | CTTTATCATT | GGCATTGTGG | GCTTTGAAGT | TGCTGGGATA |
| 101 | AATTAATATA | ATTAAATAAA | AGACTGAATT | TAATTGCAAA | AAAAAAAAAA |
| 151 | AACAATAAGT | GTGGTGAT | | | |

TCU2/1(1)

| | | | | | |
|---|---|---|---|---|---|
| 1 | AAGAAATTAT | CCAGTTATTT | ACAAGGCCAC | TGATATTTTA | AACGTCCAAA |
| 51 | AGTTTGTTTA | AATGGGCTGT | TACCGCTGAG | AATGATGAGG | ATGAGAATGA |
| 101 | TGGTTGAAGG | TTACATTTTA | GGAAATGAAG | AAACTTAGAA | AATTAATATA |
| 151 | AAGACAGTGA | TAAATACAAA | GAAGATTT | | |

TCU2/2(1)

| | | | | | |
|---|---|---|---|---|---|
| 1 | CGGGTTAATA | TTATCCTCTA | GTATAAGTGA | ATTACTAGTT | TCTCTTTATT |
| 51 | TAGACAAACA | CACACACACC | AGATAATATA | AACTTAATAA | ATTATCTGTT |
| 101 | AATGTAGATT | TTATTTAAAA | AACTATATTT | GAACATTGGT | CTTTCTTGGA |
| 151 | C | | | | |

TCU9/1(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | ACATAACAGC | TTTTATACAA | TGATAAGGAC | ATATCATTTG | TTTACAAAGA |
| 51 | AAGTCTAAAA | TTTCAAGAAC | ATTCAAAGAG | CTAACACAGT | AAAGGTCATG |
| 101 | CAAGTTCTAG | AATAGTGAAT | CATGACAGAA | CTCATTCATT | TTATCCTTTA |
| 151 | TCTCC | | | | |

TCU9/2(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | AAGTATGGGT | AGCTAAATTT | GCATTAAATT | AAAAGTACAT | ATAATGCAAC |
| 51 | ACCACTCTAC | ATCTGTATAC | CTACGAATGT | ATGTGTACTA | CACACCCTTA |
| 101 | AAATGTTTTT | CAAAGTCTTA | ATATATTAGA | ACATGTTTTC | ATTTTTTCAT |
| 151 | GGGATGTTAA | TACTATTCTA | TGATTAAGAA | AATACTAG | |

TCU10(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | AATACAGTTA | TTCTAGCTTT | TCATATTCAA | TTTGAATGAT | CAGAAAAGTA |
| 51 | TATTAGTCAC | ACAGAATTAA | ATATTTTAGA | TAGTAAGAAT | C |

TCU14(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | GAAGTGAAAG | TCAGCCCTTT | AGCTATTATT | TATTGCTTTA | TTAGAGCAGA |
| 51 | GGGAAGTGAC | ACTCATTGCC | TTCACAGAGC | TCTGCAGAAA | TATATGCACA |
| 101 | GAGTGGTCAA | TGCCAACATC | TGAGTAAGTC | TTCCAAA | |

TGO20(2)

| | | | | | |
|---|---|---|---|---|---|
| 1 | CAGAACATTA | GGATTTATTC | CTTGATTAGT | TCAAATGATT | TCAACAGCTG |
| 51 | AATTCCTTGA | GATGTGTAAG | GCAGGTTGGT | CCTTTGGATG | GACTGTAGAC |
| 101 | TGAAACTTCC | TATAACTGTA | GTGATATGTA | CACAGCTACA | TAGCAAAGTG |
| 151 | CTTCATTATG | AAAATGAAGA | A | | |

TGO20(1)

| | | | | | |
|---|---|---|---|---|---|
| 1 | CAGTGTGAGA | GTCTCATTTC | TATGCACAGT | GTTTCTCAGG | AGGATGGAGC |
| 51 | TAGTTAGCTG | TCTGTTGTCT | GTAGCCCAGC | TTGATAATGG | AACTATACAG |
| 101 | CGAAGAGACA | ATCTCTGGCA | AGTTTTTGTA | GAA | |

TGU5(C)

| | | | | | |
|---|---|---|---|---|---|
| 1 | TTAGAGTAAA | ATTCCAAATA | AATGCTTTGC | TCCAAAATTA | CACTAACCAG |
| 51 | GCTGGGTCTC | TATCATACAT | CTTCAATACC | CTCAAACCTA | GATTGTAAAG |
| 101 | TGAAAAAAGT | GATTAGCNNT | TCCATTTGTT | CATTCTGTCA | CTCACATTCT |
| 151 | TAGGCATTTT | AAGGATGAGC | AACCTTTGTT | TCAGAAAGGG | TAAGTAATTA |
| 201 | GCCCCCTGGA | GGTTACATAG | TTATAATTTA | GTCTTCAGAA | TCCGTTCGAA |

```
251   GGGNNNNGTT   ACTATTTTTA   AGATAATTAG   AACCCACCTT   GTAGCAATAA
301   AAGTTTTCTT   GTCTTTG

TGU8(2)
  1   GCGAAAGACT   AATCGAACCA   TCTAGTAGCT   GGTTCCCTCC   GAAGTTTCCC
 51   TCAGGATA

TGU9/1(2)
  1   TTAATGTTTA   AATACTACTT   TTTTTTCAAG   CTTGCCCTAG   ATACCAACTG
 51   TTTATCTAAC   ACACAATTCC   AGTGTTGCCA   AGCCTCATGC   CAATTTGAAG
101   GGAACAGCCA   AAACTTATGC   ATTCATATAA   AAAGAGTCTC   TAGGCTCTTA
151   TATCTACATT   ATAATTTTT

TGU9/2(2)
  1   GGAATAACAT   TTTTTTATGA   GGGAACCCTT   TAAAATGGAT   GCACACAGTG
 51   GCATTTTCTC   CTAGGCTCAA   AGCTGAGTAC   ACTCCCGTAA   TTTTAATAAT
101   ATTTTAGGCA   AGTCCTATGA   CAATTATACC   AACAAGTTTC   TTCAACCCCA
151   CCACCACCCC   ACCATCTCTA   TGC

TGU12(C)
  1   GGAGGAAGCT   TTATTTGGGA   AGAGTGCGGT   TCNNTCGGCC   CTGATCAGCT
 51   CTAGCCTGCC   CACCCCATCT   CAGCCAGGCG   GCTTTACTTC   TTCCTGAGCT
101   TCAGGTCTTT   CTTCTTCCTG   ATTTCCTTGG   CCAGCTCCCC   AATCAATCTC
151   CAGTACTCAT   TGAACTTGAG   CTCCGAGNCC   TGATTCACAT   CCAAGCTCTT
201   CATCTTCT

TGU13/1(C)
  1   GGATGTGGTA   GTTGATCTTT   AATGCCCATT   CTAGGTCGGA   AAAATCCATG
 51   ATCCTAACTT   TTAAGAGAAG   GTTGGTAACT   CTACTTAGGA   CTTTTTTTTG
101   TAAGAGGAAT   AATGTAGCCT   CACCCTTATC   TTTCTGGAAA   TGTTTAAACC
151   ACTGAAATAT   GGAGATCAAA   TCCAGCTTAC   ACACTGGTAA   CTCAAATACT
201   ATTTTTTTTT   TAAACTATCT   TTTCTAAACT   AATCACCCCT   CTTGTACATA
251   GAACTTTCTA   TCTCAGTGCC   AATTCTTAGA   GGTTGATGCA   AACAGCTCTC
301   CAGAGAGCCT   GTGCTATTGT   TC

TGU13/2(2)
  1   GGGGTGTACA   TTTTATTGGA   AACCTTAAAT   ACTGTTCAGA   AAGAATATAT
 51   CTTCAATCAA   GGTCTTGCCG   AGCCTACACA   GAAAAATGAA   GCTTTTTGGG
101   TTAGGGGCAA   GGATATATAC   AGTACAGAGG   ACAAAGA

TTO16/2(C)
  1   ACATTCATTA   AAGATGAACT   TTCAGCATCT   TCACTTGAAG   ATCCATCAGA
 51   TGATTCTGAG   AGGCAGGTCT   CCCCCAAAAA   TCCACCGCAT   GTATTCTTTC
101   GTTTAGAATC   TGAAAGCCTC   TTTCTTTTCA   GGCTTGATGA   CTCTTCTAAG
151   GTATTTGTTA   TGCCTCTCTT   CTGGGTTTTT   CGTTTTGCCT   TATCAAGTAG
```

201 CTNAAATTCA AACACCATGG CAANAGAAAC TGCTTCTAT

TTO20/1(C)

1 CCACCAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTCAC AGTGAGATAT
51 TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT
101 CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTTAAACCTG
151 GAGTTGATTA TACCATCACT GTGTATGCTG TCACTGGCCG TGGAGACAGC

201 CCCGCAAGCA GCAAGCCAAT TTCCATTAAT TACCGAACAG AAATTGACAA
251 ACCATCCCAG ATGCAAGTGA CCGATGTTCA AGACAACTGT TTTAATAAAA
301 GATTTACATT CCAC

TTO20/2(2)

1 TTGGTACCAC AGTCACAGAA CTGGGGGTCA TTTTCTAGAT GAAACAAACG
51 GAACAAGTTC TCTTCCAACA AAGAAATGTA CTGTAGAAAT TAATTTCCTC
101 CATGAATTTT ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA
151 AG

TTU2/1(2)

1 CTAGAACTTC CAAAGGCTGC TTGTCATAGA AGCCATTGCA TCTATAAAGC
51 AACGGCTCCT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAAGTC
101 ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCAATGC TTCTCG

TTU 2/2(C)

1 AACCAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT
51 TGTTTTCTTA TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG
101 CATGTTTTTC TTCATAGATA CCCGTTTTGG GGTTTTTGCG TCGGAAGATG
151 AAGTGCAGTT TATAGTCCTC TCCACATTTA TCTG

TTU3(1)

1 GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATTCGG
51 GAGGACCTAT TGGTGCGGGG GCTTTGTATG ATTATGGGCG TTGATTAGTA
101 GTAGTTACTG GTTGAACATT GTTTGTTGGT GTATATATTG TAATTGAGAT
151 TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG
201 AAG

TTU 5/1(2)

1 GACAAAAAAA AAAAAACAGG TTTTAAAGCT AGAAATGAAA AGCTACTTAA
51 GTATCTTAAA GGATAAGTTA CTTTATTATA CACTAGAAAC ATACACAATA
101 GCTGAAAACT TAAAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG

TTU5/2(2)

1 GCATCCATTG TACATTGTTT GGTTTGAGGT TACCATGAGG CCTGTAAATA
51 CTATCTTATA ATTTATTATT TCAACCTGAT AAAACTTAAC ACTATTTGCA
101 TAAACAAACA AACGAAAA

```
TTU9/1(1)
      1   TAAAATACTG  GTTCTTTTAT  TCTGCAATAT  TTTTAAAAAT  CACATTTTCA
     51   GCCAGGCGCA  GTTTCCCACA  CCTGTAATCC  GGCACTTTGG  GAGGCTGAGA
    101   TGGGTGGATC  ACAAGGTAGG  AGATCAAACA  TCCTGGCCAA  CATGGTGAAC
    151   CTGTTTACT
```

```
TTU9/2(2)
      1  CAAGTATGGG TAGCTAAATT TGCATTAAAT TAAAAGTACA TATAATGCAA
     51  CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCTT
    101  AAATGTTTCA AAGCTTAATA TATTAGAACA TGTTTTCATT TTCAGGGAG
```

```
TTU13(2)
      1  GGAAATACAC TAGCATGTGA GCACTGTATA TAAAGCTTGA GGTTAGGAGG
     51  TAAAATGAAA GAAATCATTT TTAACTCCTA AGATGT
```

```
TTU13(1)
      1  TGAATTAAAT GGACTCGTTG AAAGGACAAG GAGATCGGTA ATATCTCTCT
     51  AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT
    101  CTTCTTTT
```

or an analog thereof. In accordance with the invention, the term "analog" includes nucleic acids which code for the same protein sequence due to the degeneration of the genetic code, for a protein having conservative amino acids substitutions or deletions that do not eliminate the characteristical feature of this protein, or for a protein having at least about 85 %, and more advantageously at least about 90 %, in particular 95 % amino acid sequence homology.

Other embodiments of the invention provide a vector containing said DNA and a host cell containing said vector.

According to the general knowledge one skilled in the art can also use said nucleic acids of the present invention as a hybridization probe to detect the corresponding genes in an organism or in a sample from on organism or gene mutations thereof.

Therefore, an additional embodiment is a method for isolating a gene which can be induced or repressed by treating chondrocytes that contain this gene by IL-1β containing the steps:

(a) hybridizing a DNA of the present invention under stringent preferably high stringent conditions against DNA or RNA containing said gene, preferably DNA or RNA isolated originally from chondrocytes, in particular human chondrocytes; and

(b) isolating this gene by methods known to a skilled person in the art.

According to the present invention the term "stringent conditions" means hybridization conditions comprising a salt concentration of 4 x SSC (NaCl-citrate buffer) at 62-66°C, and "high stringent conditions" means hybridization conditions comprising a salt concentration of 0,1 x SSC at 68°C. The length of the probes are 6-100, preferably 10-40, in particular 12-25 nucleic acids long.

Yet another embodiment is a process for expressing a gene isolated according to the above-described process containing the steps:

(a) cloning said gene into a suitable expression vector such as the pET series (Studier et al., 1990. Methods in Enzymology 185, 60) for procaryotic expression or the vector CDM8 for mammalian expression (Aruffo and Seed, 1987. Proc. Natl. Acad. Sci. USA 84, 8573) or any other expression system known to one skilled in the art; and

(b) expressing said gene in a suitable host cell such as BL21 series (Studier et al., 1990, supra) for procaryotic expression or COS, cells for mammalian expression (Aruffo and Seed, 1987, supra) or any other expression system known to one skilled in the art;

or a method for producing a protein containing the steps:

(a) culturing a suitable host cell, in particular the above mentioned, containing a vector, in particular an expression vector such as the vectors mentioned above which contains a DNA or a gene of the present invention; and

(b) isolating the expressed protein for example by ultrafiltration, precipitation with chaotropic agents such as urea or column chromatography on e.g. ion exchange chromatography columns as detailed in Ausubel et al. 1994 (supra).

A further embodiment is a diagnostic aid containing a DNA or parts thereof or a gene or parts thereof of the present invention. In particular, quantification of the genes can be achieved on the RNA level by Northern blotting with gene specific probes of the present invention or with gene specific primers in a PCR reaction. Such primers can be synthetically produced using the DNA sequences of the present invention or the sequences of the corresponding genes. Therefore, said nucleic acids are useful for the diagnosis of IL-1β related diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

These nucleic acids can also be used to evaluate the expression of certain genes in small cartilage biopsies and to use these ultimately as disease-specific markers and/or as predictive markers for disease progression of e.g. osteoarthritis. The hybridization conditions can be the same as described above.

Said nucleic acids, however, can also be used for the therapy against the diseases mentioned or for the production of a pharmaceutical.

Therefore, another embodiment of the present invention is also the use of said nucleic acids for the production of a pharmaceutical. For example, as described by Uhlmann & Peyman (Chem. Rev. (1990), 90, 543), Milligan et al. (J. Med. Chem. (1993), 36, 1923) or Stein & Cheng (Science (1993), 261, 1004) such nucleic acids can be used as antisense oligonucleotides or triple helix forming oligonucleotides for the inhibition of gene expression. This is in particular useful if such a disease is caused by the overproduction of a gene product which is directly or indirectly regulated by IL-1β in chondrocytes. The nucleic acids can additionally be modified in order to increase e.g. the stability against nucleases as described e.g. in the literatures mentioned above.

Finally, also the gene product itself produced by a method of the present invention can be used as a pharmaceutical.

In the following the invention is in particular described by the examples and tables:

Description of the Tables

Table 1 gives an overview on used primers and the complexity of the detected differences in expression.

Table 2 summarizes the result of the sequencing of differentially displayed PCR products after their elution from the sequencing gel, reamplification and subcloning into the pCRII vector. The sequences of TAU1/1(1) and TAU1/1(2) are 100 % identical to human osteopontin cDNA, the sequence of TTU2/2 is 97.2 % identical to human calnexin. bp = base pairs, IL-1 = Interleukin-1 stimulation, Stat. sig. score = statistical significance score: a feature of the BLAST database searching program. This score is determined using an implementation of Karlin's significance formula (Karlin, S. and Altschul, S.F. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc. Natl. Acad. Sci. USA, 87:2264-2268), which calculates the Poisson probability that the observed sequence similarity will occur by chance based on the size and composition of the sequence database as well as on the size and quality of the match. The smaller this number, the more it is likely to see sequence similarities.

Examples

Cell culture

Articular cartilage specimen were obtained from two patients (male 65 years old and female 73 years old) undergoing total joint replacement surgery for osteoarthritis. None of these individuals had received treatment by radiation or chemotherapy. Articular cartilage slices were aseptically dissected from both femoral condyles, tibia plateaus and pattellae and subjected to sequential enzymatic digestion with pronase and collagenase as described (Häuselmann HJ et al. 1992, Matrix 12, 116-129) Since it is known that the alginate gel suspension system retains the chondrogenic phenotype [Lohmander LS et al. 1992, Trans. Orthop. Res. Soc. 17, 273.] $4 \times 10^6$ chondrocytes were suspended in low viscosity alginate ($4 \times 10^6$ cells / ml 1,25 % w/v alginate in an isotonic buffered solution) and expressed through a 22gauche needle into 102 mM CaCl solution to form cell entrapping beads which are 1,5-3 mm in diameter and spherical. Alginate beads containing a total number of $2 \times 10^7$ cells were fed daily for the first three days with medium F12 / DMEM (50/50)

and 10 % fetal calf serum (Sigma) with 25 µg / ml ascorbate and 50 µg / ml gentamycin and were then subdivided into two populations for further three culture days in the presence or absence of 5U / ml rh IL-1β (Genzyme). For cell recovery, alginate beads were finally dissolved into dissolution buffer (55 mM sodiumcitrate, 30 mM EDTA, 0,15 M NACl) and placed at room temperature for 10 min. Viability was checked by eosin-red exclusion and cell number was determined.

Primer syntheses

Arbitrary oligodecamer primers OPA6 to OPA10, OPA16 to OPA20 and degenerate anchored oligo-dT primers ($T_{12}VN$) were synthesized using the 392 DNA synthesizer (Applied Biosystems) and purified by denaturing polyacrylamid gel electrophoresis. Some oligodecamer primers, U1 to U15 were purchased from Biometra (Göttingen, FRG).

List of all degenerate 3' oligo dT-primers [$T_{12}VN$] used for DDRT-PCR:

| Primer | Sequence 5' to 3' |
|---|---|
| $T_{12}VA$ | 5'-T T T T T T T T T T T T V A-3' |
| $T_{12}VA$ | 5'-T T T T T T T T T T T T V T-3' |
| $T_{12}VA$ | 5'-T A T T T T T T T T T T V G-3' |
| $T_{12}VA$ | 5'-T T T T T T T T T T T T V C-3' |
| V = dA, dG, dC; N = dA, dT, dG, dC | |

List of all arbitrary 5' oligodecamer primers used for DDRT-PCR:

| Primer | Sequence 5' to 3' |
|---|---|
| OPA 6 | G G T C C C T G A C |
| OPA 7 | G A A A C G G G T G |
| OPA 8 | G T G A C G G G T G |
| OPA 9 | G C G T A A C G C C |
| OPA 10 | G T G A T C G C A G |
| OPA 16 | A G C C A G C G A A |
| OPA 17 | G A C C G C T T G T |
| OPA 18 | A G G T G A C C G T |
| OPA 19 | C A A A C G T C G G |
| OPA 20 | G T T G C G A T C C |
| U1 | T A C A A C G A G G |
| U2 | T G G A T T G G T C |
| U3 | C T T T C T A C C C |
| U4 | T T T T G G C T C C |
| U5 | G G A A C C A A T C |
| U6 | A A A C T C C G T C |
| U7 | T C G A T A C A G G |
| U8 | T G G T A A A G G G |
| U9 | T C G G T C A T A G |
| U10 | G G T A C T A A G G |
| U11 | T A C C T A A G C G |
| U12 | C T G C T T G A T G |
| U13 | G T T T T C G C A G |
| U14 | G A T C A A G T C C |
| U15 | G A T C C A G T A C |

RNA isolation and cDNA synthesis

Total RNA from cultured articular chondrocytes was prepared according to a single step method Chomczynski and Sacchi (Chomczynski P & Sacchi N 1987, Anal. Biochem. 162, 156-159) and incubated with 10 U RNasefree DNaseI (Gibco, Eggenstein, FRG) for 30 min at 37°C to remove chromosomal DNA contamination of RNA. After extraction with phenol/choroform (3:1), the supernatant was ethanol precipitated in the presence of 0.3 M NaOAc and RNA was redissolved in DEPC treated water. 0,4 µg total RNA was then reverse transcribed using 200 U M-MLV (Moloney murine leukemia virus) reverse transcriptase (Gibco, Eggenstein, FRG) in a 40 µl reaction volume containing 50 mM Tris-HCl (pH 8,3), 75 mM KCl, 10 mM DTT, 3 mM $MgCl_2$, dNTP mix (dATP, dTTP, dCTP, dGTP) of 200 µM each, 40 U RNase inhibitor (Boehringer Mannheim, FRG) and 2,5 mM degnerate oligo-dT primer ($T_{12}VN$) at 37°C for 1 h. Reactions were terminated by heating for 5 min at 95°C.

PCR amplification

cDNAs were amplified in a DNA thermal cycler (Perkin Elmer, model 480) in 20 µl PCR reactions containing 2.5 µM of the original $T_{12}MN$-primer used in cDNA synthesis in combination with 0.5 µM arbitrary upstream primer, dNTP mix (dGTP, dCTP, dTTP) of 0,5 µM each, 10 µCi $\alpha$-[$^{35}$S]dATP (1000 Ci/mmole, 10 mCi/ml), 10 mM Tris-HCl (pH 8.3) 50 mM KCl, 1,5 mM MgCl, 0,001 % gelatin and 2.5 U AmpliTaq DNA polymerase. Light mineral oil was overlaid and thermal cycling was performed as follows: 94°C for 30 seconds, 40°C for 2 min and 72°C for 30 seconds for 40 cycles followed by 5 min postextension at 72°C. AmliTaq DNA polymerase was purchased from Perkin-Elmer (Weiterstadt, FRG) and $\alpha$-[$^{35}$S]dATP was obtained from Amersham-Buchler (Braunschweig, FRG). After addition of 5 µl stop buffer (95 % formamide, 20 mM EDTA, 0,05 % bromphenolblue and 0,05 % xylene cyanol) radiolabeled PCR-fragments were then displayed on 6 % acrylamide/7 M Urea high resolution sequencing gels of 35 x 43 cm in size; dried gels were exposed to X-ray film (Kodak X-OMAT) and exposed for 48 h, which allows rapid identification of differentially expressed genes by side by side comparison of DDRT-PCR band patterns.

Elution, reamplification and cloning of PCR fragments

PCR fragments identified as differentially expressed bands were cut from acrylamide gels, transferred into Eppendorf tubes and rehydrated for 10 min with 100 µl 10 mM Tris-HCl and 1 mM EDTA at room temperature. After boiling the gel slice for 15 min, the PCR fragment was recovered by ethanol precipitation in the presence of 0.3 M NaAc and 20 µg glycogen as a carrier and redissolved in 10 µl sterile water. 5 µl of this volume was used for reamplification by PCR using appropriate primers and conditions described above except for dNTP concentration of 20 µM and no radioisotope. The reamplified PCR product was visualized by electrophoresis on a 2 % agarose gel and eluted from the gel by ultrafiltration using Ultrafree MC-filters (Millipore). Purified PCR fragments were then cloned into the pCRII-vector (Invitrogen, De Schelp, NL) by the TA cloning method (Kovalic D et al. 1991, Nucleic Acids Research 19, 4640), which allows in-vitro transcription and sequencing from the plasmid.

Sequencing

Plasmid DNA sequencing of subcloned PCR fragments with either SP6(2) or T7(1) primer was carried out using the chain-termination DNA sequencing method, as described by Sanger et al. (Sanger F et al. 1977, Proc. Natl. Acad. Sci. USA 74, 5463-5467.).

Sequence analysis

The sequence analysis revealed the sequences of cDNA clones TAO8/2(2), TAO16/1(2), TAO16/2(2), TAO17(c), TAO19(c), TAU1/1(2), TAU1/1(1), TAU1/2(2), TAU7/1(2), TAU7/1(1), TAU7/2(c), TAU10(1), TAU12/1(2), TAU12/1(1), TAU12/2(1), TAU12/3(2), TAU12/3(1), TAU13/1(1), TAU13/3(2), TAU13/3(1), TCO16/1(c), TCO16/2(c), TCO17(c), TCO18(c), TCU2/1(1), TCU2/2(1), TCU9/1(2), TCU9/2(2), TCU10(2), TCU14(1), TCU14(2), TGO20(2), TGO20(1), TGU5(c), TGU8(2), TGU9/1(2), TGU9/2(2), TGU12(c), TGU13/1(c), TGU13/2(2), TTO16/2(c), TTO20/1(c), TTO20/2(2), TTU2/1(2), TTU2/2(c), TTU3(1), TTU5/1(2), TTU5/2(2), TTU9/1(1), TTU9/2(2), TTU13(2), TTU13(1) disclosed on pages 7 to 14 of the specification.

Searching for homology between subcloned PCR fragments and sequences already listed in one of the DNA databases (GenBank or EMBL database) was performed using the FASTA program developed by Pearson and Lipman (Pearson W & Lipman DJ 1988, Proc. Natl. Acad. Sci. USA 85, 2444-2448) included in the GCG software package (Genetics Computer Group, Madison, USA).

Northern-blot analysis

Cell culture and isolation of RNA was performed exactly as described above. 10 μg of total RNA from both IL-1β stimulated or not stimulated chondrocytes were denatured by heating at 65°C for 10 min in a solution of 50 % formamide, 20 mM MOPS and 2.2 M formaldehyde, separated through a 1 % agarose gel containing 2.2 M formaldehyde in 1 X MOPS and transfered to positively charged nylon membrane (Amersham) by standard blotting procedures [Maniatis et. al 1992]. After UV crosslinking, the blots were prehybridized for 1 h in rapid-hyb-buffer (Amersham) at 65°C. A 330 bp cDNA corresponding to nts 61 to 390 of human osteopontin cDNA (GenBank J04765) and a 340 bp cDNA corresponding to nts 881 to 1220 from human calnexin (GenBank M94859) were radiolabeled for hybridization with α-[$^{32}$P]dCTP (3000 Ci/mmol, 10 mCi/ ml) using random nonamer primers (Amersham) up to a specific activity of ~ 1,5 x $10^9$ dpm / μg DNA. Hybridization was performed for 2,5 h at 65°C in prehybridization solution with 2 ng / ml of labeled probe added. The blot was subsequently washed in 2 X SSC, 0.1 % SDS at 37°C for 15 min (1 X SSC = 0,15 M NaCl, 0.015 M sodium citrate, pH 7,0), followed by two sucessive washes with 1 X SSC , 0.1 % SDS at 65°C for 10 min respectively. If necessary, a final high stringency wash was performed with 0.1 X SSC , 0.1 % SDS at 65°C for 15 min. The blots were then analysed by autoradiography using Kodak X-Omat films at -80°C with intensifying screeens for 2-7 days and intensity of bands was quantified with a phosphorimager (Biorad, model GS-250). All blots were stripped with boiling 0.5 % SDS solution and reprobed with labeled β-actin to demonstrate equal loading of RNA in each lane.

Northern hybridisations (Results)

Fragment TAU7/2(c), identical to TSG-6, was differentially upregulated in IL-1 stimulated cells. This is in concordance with Lee et al. (1992) which reported for TSG-6 a TNF-α and IL-1 mediated upregulation. Fragment TAU1/1, identical to human osteopontin and fragment TTU2/2, identical to human calnexin, both were weaker expressed in IL-1 stimulated chondrocytes compared with the unstimulated cells. To validate our differential display data, we performed Northern analyses of Osteopontin and calnexin expression in IL-1 stimulated and unstimulated chondrocytes originating from a third patient. Both messages were again downregulated. A phosphorimager quantification revealed an osteopontin downregulation by 79% and a calnexin downregulation by 40% in the RNA population from chondrocytes of the third

patient.

Table 1: Current results of differential display reverse transcriptase PCR (DDRT-PCR) to reveal diffential gene expression by chondrocytes with and without IL-1ß

Overview on used primers and number of analysed bands

DDRT-PCR primercombination

| 3'-Oligo dT-primer (downstreamprimer) | 5'-Oligodecamer (upstreamprimer) | putative differential expressed genes by side by side comparison DDRT-PCR band pattern | reproducibility of DDRT-PCR band pattern from first to second, third or fourth DDRT-PCR (same patient[1]) | (other patient[2]) | eluted from gel and reamplified in PCR | chloned into pCRII vector by TA cloning method verified by PCR | PCR-fragment sequenced using SP6 or T7 promoter |
|---|---|---|---|---|---|---|---|
| $T_{12}M^*A$ | OPA 6 - OPA 10 | 25 out of ~ 4000 bands | 7 | not done | 6 | 1 | 1 |
| $T_{12}M^*T$ | OPA 16 - OPA 20 | 19 out of ~ 4000 bands | 13 | 9 | 12 | 12 | 12 |
| $T_{12}M^*G$ | U 1 - U 5 | 31 out of ~ 4000 bands | not done | 11 | 11 | 10 | 10 |
| $T_{12}M^*C$ | U 6 - U 10 | 27 out of ~ 4000 bands | not done | 13 | 12 | 11 | 11 |
| | U 11 - U 15 | 21 out of ~ 4000 bands | not done | 11 | 11 | 10 | 10 |
| total 4 x = 100 combinations | 25 | total 123 | total | 55 | total 52 | total 44 | total 44 |

* means threefold degeneracy where M may be dA, dG or dC

1 patient female 73 years old diagnosis gonarthosis

2 patient male between 65-75 years old

theoretical consideration:

Suggesting that an arbitrary upstream primer detects 3 % of the total RNAs (Liang 1994), then 97 % of the total mRNAs will not be detected, i.e. with 25 arbitraty oligodecamerprimer and the four degenerate $T_{12}VN$ primers, about half of the mRNAs would be seen ($P=1-(0,97)^n=1-(0,97)^{25}=53,3$ %) in 100 lanes of high resolution sequencing gels.

Table 2 **IL-1 mediated differentially displayed cDNA fragments of human articular chondrocytes**

| Fragment | bp | IL-1 | Features | Stat.sig.score |
|---|---|---|---|---|
| TAO 8/2(2) | 275 bp | + | 146 bp sequenced, no homology found | 0.999 |
| TAO 16/1(2) | 450 bp | + | 80 bp sequenced, no homology found | 0.69 |
| TAO 16/2(2) | 200 bp | + | 115 bp sequenced, no homology found | 0.04 |
| TAO 17(c) | 412 bp | + | 412 bp sequenced, no homology found | 0.016 |
| TAO 19(c) | 209 bp | -- | 209 bp sequenced, no homology found | 0.99 |
| TAU 1/1(1,2) | 450 bp | -- | 100 % sequence identity to human **osteopontin** cDNA in 303 bp overlap (303 bp seq.) | 1.2 x 10-101 |
| TAU 1/2(2) | 430 bp | + | 188 bp sequenced, no homology found | 0.82 |
| TAU 7/1(1,2) | 500 bp | + | 87 % sequence identity to human cDNA clone **c-1sd02** in 125 bp overlap (235 bp seq.) | 8,1 x 10-33 |
| TAU7/2(c) | 202 bp | + | 99.5 % sequence id to human **TNF stimulated gene-6** in 202 bp overlap | 4.8 x 10-76 |
| TAU 10(1) | 400 bp | + | 181 bp sequenced, no homology found | 0,9997 |
| TAU 12/1(1,2) | 470 bp | -- | 319 bp sequenced, no homology found | 3.3 x $10^{-14}$ |
| TAU 12/2(1) | 390 bp | -- | 155 bp sequenced, no homology found | 0.0078 |
| TAU 12/3(1,2) | 250 bp | -- | 95 % sequence identity to human cDNA clone **HRBBA21** similar to S10 in 158 bp overlap (162 bp seq.) | 1.0 x $10^{-28}$ |
| TAU 13/1(1) | 600 bp | + | 145 bp sequenced , no homology found | 0.12 |
| TAU 13/3(1,2) | 500 bp | -- | 439 bp sequenced, no homology found | 0.33 |
| TCO 16/1(c) | 241 bp | + | 241 bp sequenced, no homology found | 2.4 x $10^{-7}$ |
| TCO 16/2(c) | 230 bp | + | 230 bp sequenced, no homology found | 4.3 x $10^{-5}$ |
| TCO 17(c) | 169 bp | + | 169 bp sequenced, no homology found | 0.49 |
| TCO 18(c) | 168 bp | + | 168 bp sequenced, no homology found | 1.3 x $10^{-6}$ |
| TCU 2/1(1) | 400 bp | + | 178 bp sequenced, no homology found | 0,66 |
| TCU 2/2(1) | 210 bp | + | 151 bp sequenced, no homology found | 0.0074 |
| TCU 9/1(2) | 430 bp | + | 99 % sequence identity to human cDNA clone **131036 3'** in 155 bp overlap (155 bp seq.) | 7,2 x $10^{-58}$ |
| TCU 9/2(2) | 320 bp | -- | 188 bp sequenced, no homology found | 0,22 |
| TCU 10(2) | 320 bp | -- | 100 % sequence identity to human cDNA clone **26518 3'** in 85 bp overlap (91 bp seq.) | 2,9 x $10^{-28}$ |

| Fragment | bp | IL-1 | Features | Stat.sig.score |
|---|---|---|---|---|
| TCU 14(1,2) | 280 bp | + | 99.3 % sequence identity to human cDNA **HL60 3'directed MboI** in 249 bp overlap (249 bp seq.) | $3,5 \times 10^{-51}$ |
| TGO 20(1,2) | 300 bp | + | 304 bp sequenced, no homology found | 0.95 |
| TGU 5(c) | 317 bp | + | 317 bp sequenced, no homology found | 0.088 |
| TGU 8(2) | 320 bp | + | 100 % sequence identity to human **28S rRNA** in 58 bp overlap (58 bp seq.) | $1.4 \times 10^{-16}$ |
| TGU 9/1(2) | 280 bp | + | 169 bp sequenced, no homology found | 0,55 |
| TGU 9/2(2) | 220 bp | -- | 100 % sequence identity to human cDNA clone **12A10B** in 100 bp overlap (173 bp seq.) | $4.0 \times 10^{-36}$ |
| TGU 12(c) | 208 bp | -- | 87 % sequence identity to human cDNA clone **113442 3'** in 208 bp overlap | $5.5 \times 10^{-63}$ |
| TGU 13/1(c) | 322 bp | + | 322 bp sequenced, no homology found | $6.9 \times 10^{-13}$ |
| TGU 13/2(2) | 300 bp | -- | 94.9 % sequence identity to human **F1 ATPase β-subunit** in 137 bp overlap (137 bp seq.) | $2.3 \times 10^{-43}$ |
| TTO 16/2(c) | 239 bp | + | 97.5 % sequence identity to human **ERCC5** in 239 bp overlap (239 bp seq.) | $9.3 \times 10^{-88}$ |
| TTO 20/1(c) | 314 bp | + | 100 % sequence identity to human **fibronectin** in 314bp overlap (314 bp seq.) | $1.9 \times 10^{-121}$ |
| TTO 20/2(2) | 400 bp | + | 152 bp sequenced, no homology found | 0.035 |
| TTU 2/1(2) | 300 bp | -- | 100 % sequence identity to human cDNA clone **118470 5'** in 146 bp overlap (146 bp seq.) | $2,1 \times 10^{-36}$ |
| TTU 2/2(c) | 184 bp | -- | 99 % sequence identity to human **calnexin** in 184 bp overlap (184 bp seq.) | $2.3 \times 10^{-64}$ |
| TTU3(1) | 400 bp | + | 97 % sequence identity to human **NADH-DH mtDNA subunit** in 203 bp overlap (203 bp seq.) | $8.6 \times 10^{-69}$ |
| TTU 5/1(2) | 300 bp | -- | 147 bp sequenced, no homology found | 0.0065 |
| TTU 5/2(2) | 270 bp | -- | 118 bp sequenced, no homology found | 0,035 |

| Fragment | bp | IL-1 | Features | Stat.sig.score |
|---|---|---|---|---|
| TTU 9/1(1) | 350 bp | + | 94 % sequence identity to human cDNA clone **83764 3'** in 159 bp overlap (159 bp seq.) | $5,9 \times 10^{-23}$ |
| TTU 9/2(2) | 320 bp | -- | 149 bp sequenced, no homology found | 0,22 |
| TTU 13(1,2) | 350 bp | + | 194 bp sequenced, no homology found | 0,57 |

Thus, the 44 identified fragments can be subdivided as follows:

1) 2 fragments with sequence homologies to known human genes with known roles in IL-1 mediated processes:

| | |
|---|---|
| TAU 7/2 | identical with human TNF-stimulated gene-6 |
| TTO 20/1 | identical with human fibronectin |

2) 6 fragments with sequence homologies to known human genes, whose function in IL-1 mediated processes can be speculated:

| | |
|---|---|
| TAU 1/1 | identical with human osteopontin |
| TGU 8 | identical with human 28S ribosomal RNA gene |
| TGU 13/2 | identical with human F1 ATPase β-subunit |
| TTO 16/2 | identical with human ERCC5 |
| TTU 2/2 | identical with human calnexin |
| TTU 3 | identical with human NADH-DH mtDNA subunit |

3) 9 fragments with sequence homologies to human genes, identified in human geneome sequencing projects:

| | |
|---|---|
| TAU 7/1 | identical with human cDNA clone c-1sd02 |
| TAU 12/3 | identical with human cDNA clone HRBBA21 |
| TCU 9/1 | identical with human cDNA clone 131036 3' |
| TCU 10 | identical with human cDNA clone 26518 3' |
| TCU 14 | identical with human cDNA clone HL60 3' directed MboI |
| TGU 9/2 | identical with human cDNA clone 12A10B |
| TGU 12 | identical with human cDNA clone 113442 3' |
| TTU 2/1 | identical with human cDNA clone 118470 5' |
| TTU 9/1 | identical with human cDNA clone 83764 3' |

4) 27 fragments without sequence homologies to known human genes The detection of TSG-6 and fibronectin, both genes known to be upregulated by IL-1, points to the importance of those other cDNA fragments in the light of IL-1 mediated processes. Those genes very likely play roles in degenerate joint diseases, including rheumatoid and osteoarthritis and with this are interesting candidates as markers for clinical studies or as drug targets for pharmacological intervention.

## Claims

1. Use of osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β a mediated diseases of connective tissues, in particular osteoarthritis.

2. Diagnostic aid for the diagnosis of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

3. Pharmaceutical for the prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

4. Use of calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis.

5. Diagnostic aid for the diagnosis of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.

6. Pharmaceutical for the prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.

7. Use of TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis.

8. Diagnostic aid for the diagnosis of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

9. Pharmaceutical for the prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

10. DNA containing a DNA selected from the group consisting of

```
TAO8/2(2)
      1  CCAAGTTTTT CCAGCAACCC CAAGGGAATA CAGGGAGATC AATGCACCCA
     51  AAATGGGAAA AGAAAAATAC TTCGATGCAA TGAAACAAAG CCTTTTTCCG
    101  TTCAGTTTCC ATAATTCAGT GGTCAGTTTT AAGGCTGCCA CTTGGG

TAO16/1(2)
      1  GACACGAACA CCACATATTT TTATTGGAGG CCCCATGGCT CCTTGGAAGC
     51  CATTTTGGAA CCAAGGGGAC CCACCTTTTT

TAO16/2(2)
      1  CTAAATATAT TCTCTAACAA GTTAATCTCT TTCAAATCTA TAGATAAAAC
     51  TAAAAGGATA AGGAACCAAG GTTTAACCGA CCTAGCCAAT TATGGCAATC
    101  ATACTTGCTT TTTAG

TAO17(C)
      1  CATGAAATAT TTCTTGAGGT AATAAGCTTT TACCAAGCTT ATATTTTTGG
     51  GCAATTCAGT TACAATGAGA AAAAAACACA CCAAAAGACC AAAAATTTTA
    101  AAAACTCACT TTTCTTGCAA TCATAGACAT TTGCATTATT ATAGAACATT
    151  CAAACAAGTT AGGTGGATAA TTATTGTCTA TAGATAAATA CGATGCAATT
    201  TTAATAAGAA TTTGAAGAAT GACATTAAAT GCTGTCTGAA GCCTTTGTAT
    251  TTTTTAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA
    301  AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTTAAC
```

351 AGATATGATG ACAGNAAAAA TTGATTGCAT ATCTTTTTGC ACTAAAACTT
401 TTATATTTAT TT

TAO19(C)
1 AGAGCAGGGG TATTTCNCGG TTCATACCGC CATGGCTTAA GAAGCAAAAG
51 TCATATACCT TAGTAGTGGC AAAGATNGAG GAGATAAAAA AGAGCCTACC
101 CAAGCTGTTG TTGAAGAACA GGTCTTAGAT AAAGAGGAAC CCTTCCAGAA
151 GNACAGAGAC AGGCTAAGGG TGATGCTGAG GAAATGGCTC AGAAGAAACA
201 AGAGATTAA

TAU 1/1(2)
1 CTAAATGCAA AGTGAGAAAT TGTATTTTTT CTCCTTTTAA TTGACCTCAG
51 AAGATGCACT ATCTAATTCA TGAGAAATAC GAAATTTCAG GTGTTTATCT
101 TCTTCCTTAC TTTTGGGG

TAU 1/1(1)
1 ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC
51 ATCCCCGTTT CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG
101 GAAGGACAGT TATGAAACGA GTCAGCTGGA TGACCAGAGT GCTGAAACCC
151 ACAGCCACAA GCAGTCCAGA TTATATAAGC GGAAA

TAU1/2(2)
1 CCGGAATGGG GAGCAAACTA TAAGAACCGG GACCAGTTTC CTCTCTTTGT
51 GCCCTAGTTC CCCCTCCTTT GTATACACCC TCCATCCTGA ATAGACTCTG
101 GTTCTCAGCG TAACACCGAC AACATTCAAT CCTGTAGAGA AACAAATGTT
151 AGCTCAGAAG GACACAGCCT TTGAATCATC AGAGAGTT

TAU 7/1(2)
1 GTTAAGAATA ACTAAATAAA AGTTTTAATT AATTTAGGAA TATAAAAAAC
51 TATTAACATT TAATTTTATA ACTGTATCTG CCAAGCAACT TTAAATATAA
101 TTTATTTACC

TAU 7/1(1)
1 CACGCAATGT GAAATAGGCA CATAGGAAGA ATGGGGAAAC CATCCCCTCA
51 AGCATTTATC CTTTGAGTTA CAAGCAATCC AATTACACTC TTTTAGTTAT
101 TTTTAAATGT ACAGTTAGGT TATTA

TAU 7/2(C)
1 CCTTGAAGAT GACCCAGGTT NCTTGGCTGA TTATGTTGAA ATATATGACA
51 GTTACGATGA TGTCCATGGC TTTGTGGGAA GATACTGTGG AGATGAGCTT
101 CCAGATGACA TCATCAGTAC AGGAAATGTC ATGACCTTGA AGTTTCTAAG
151 TGATGCTTCA GTGACAGCTG GAGGTTTCCA AATCAAATAT GTTGCAATGG
201 AT

TAU10(1)

```
  1  GGAGATGACA TTTGCTTTGG GCAGAGGCAG CTAGCCAGGA CACATTTCCA
 51  CTATAATTTT ACAAAGTTAA ATTTATAAGC TAGCATTAAG TAAAGTGAAG
101  TTCCAGCTCC CTTGCTAAAA ATAACTAGAG GTAATAATTG GTATTCAGGT
151  AACTCATTTA CATCATAATG TGTTGTGAAA A
```

TAU12/1(2)

```
  1  TATAAAATAT AAATTATATT ATAAATCATG TATTATTTAT AAAATTATAT
 51  TATAAATTTA TAAAAATATA AATTATATTT TAGGCTTAAT GTATAAGGAA
101  TATAAATTAT TAATAAGCAT ATGA
```

TAU 12/1(1)

```
  1  TGTAATTAAC TGTNCTTGTA GGTCTGTCTT TTATACATGT GTGAGTTTTT
 51  CTTTACAATA GATTCCTAGC ATTGGGATTG CTAGGTCAGA TGGTATGCAC
101  ATTTGACATT TTGATTGATA GCACCAGATT GCTTTGTTAA AAAATTTTNN
151  TTTATAGTTT ACATTATCTT TGTACAATAG ATGTTCTCTT TCGAC
```

TAU 12/2(1)

```
  1  GGGAAGTGAA TTGAAAATAC TTCTTTNTCA ACATAATTTT NGGGTTTTGA
 51  AATTGTGTTT GGGTTTTCAG GAAATTGGTG GTAATCTTGT ATTAGCTGAA
101  AAAAAGTGAA TTTTAAAATT CTCAGTGAAG AAGCAAATGA TTTATTTTTC
151  ATAGA
```

TAU12/3(2)

```
  1  TGTTCTGGTA ACTGTTCTAA TTGTGTCTTT GTTACTTCCA GTGCAACCCT
 51  TTCAGGTAAG
```

TAU12/3(1)

```
  1  CTAAAGAACT TGGTATCTCT ATTAAAGCAC ACGAACCTCC AAGGAAAATA
 51  GAGCGATTTA CTCTTCTCAT ATCAGTGCAT ATTTATAAGA AGCACGGAGT
101  CA
```

TAU13/1(1)

```
  1  AGTCATCAAT TCCTTTTTAT CTGTAATTAC ACATTTGTTT TTATTTCAAA
 51  GTAATTATAA GGTGTTATAT TGCATATAAT CAGAAAACTA AATGGAAATA
101  AAATTTTAGT AAGCCCGGCC CCTTTGACCG ATACAGAAAA CTTGA
```

TAU 13/3(2)

```
  1  TATATGGCAG TCTAAAGCAT CAAAGATTTG CATCAACATC TTTCATTTTA
 51  GACATCTCCT TGCAATGTAA AATATCATGT ATCAACAACA TCTGGTGCAA
101  ATCCATGAGT CTAACTCGAC ATTCATCTTA GCTCGATTAT TATTCCTTCG
151  TACAGTCGAT GTAAACAATA CAGAAAGAGG ATTATTAAGA ACAGTTT
```

TAU 13/3(1)

```
  1  ATTCATGAAA TGGTCTATAT GCATGATATT GTAAATTCGG ACTCGAAACC
 51  GAAACCAAGG ATTCCGTTAC AAAAATTCCT TAATGCTGAG AATGTTCTCA
101  CGCAAACAAC ATCATGGACA TTAAATTCAA GATATGTGAA TGTTAATTCT
151  GTCAATAAAG TCAACGTAAA GAGTAAAGTT AAAAACAGTT ATATCTNNNC
201  TGTCAATGAT GAGTTTAGTT TAACAGATGA TGAATCAATT CT
```

TCO 16/1(C)

```
  1  CAAAGTGTTT TTGGTTTTGA GAGAGAGAGA GATTGAGAGA CAGAGAGAGA
 51  GAGAGAAACC AAGGGATCAT GATAGTTATA GTCAAATACG AGGTTGGATT
101  ATCTTTTGAA AATGTGTTGG TTCTGTGATA CAAGAGGAAG CTAAGACATA
151  TCGTGGAAAC ATCTCCCCCC TCCACCTTAA TATCAAGAAC AAATTGTGGA
201  ATCTAATGTT AATGAGAAGT AGTTCCCCAC TGTGTCAGAT G
```

TCO16/2(C)

```
  1  NCATCTGACA CAGTGGGGAA CTACTTCTCA TTAACATTAG ATTCCACAAT
 51  TTNNNCTTGA TATTAAGGNN NNNNNGGGAG ATCGTTTCAC GATATCGTCT
101  TAGCTTCCTC TTGTATCACA GAACCAACAC ATTTCAAAAG ATAATCCTTC
151  CTCNNTTTGA CTATAACTAT CATGATCCCT TGGTTCTCTC TCTCTCTCTG
201  CTCTCTCATC TCTCTCTCTC TNAAAACNAA
```

TCO17(C)

```
  1  ACAGTAGTTA GGAGTTTCTT TACTTACAAA ATCACTGGAA ATGATTAAAT
 51  TGCTTTTCCC CCTCCCCAGA GGTGCATTTT TCTTATTTCC ATATAGTAAA
101  GTTGAGCTTT TACAGTGCAT AATGTGACAT TTGGAATGCT TATCAACTGC
151  ATGTAAACAT TAATAACCT
```

TCO18(C)

```
  1  GTAAATGGTA TTANNNGCTG AAGAAAAAAA ATTTTTCAAG ACCTCTGTTT
 51  TTTAACTGAA CTTTATCATT GGCATTGTGG GCTTTGAAGT TGCTGGGATA
101  AATTAATATA ATTAAATAAA AGACTGAATT TAATTGCAAA AAAAAAAAAA
151  AACAATAAGT GTGGTGAT
```

TCU2/1(1)

```
  1  AAGAAATTAT CCAGTTATTT ACAAGGCCAC TGATATTTTA AACGTCCAAA
 51  AGTTTGTTTA AATGGGCTGT TACCGCTGAG AATGATGAGG ATGAGAATGA
101  TGGTTGAAGG TTACATTTTA GGAAATGAAG AAACTTAGAA AATTAATATA
151  AAGACAGTGA TAAATACAAA GAAGATTT
```

TCU2/2(1)

```
  1  CGGGTTAATA TTATCCTCTA GTATAAGTGA ATTACTAGTT TCTCTTTATT
 51  TAGACAAACA CACACACACC AGATAATATA AACTTAATAA ATTATCTGTT
101  AATGTAGATT TTATTTAAAA AACTATATTT GAACATTGGT CTTTCTTGGA
151  C
```

TCU9/1(2)

```
  1  ACATAACAGC TTTTATACAA TGATAAGGAC ATATCATTTG TTTACAAAGA
 51  AAGTCTAAAA TTTCAAGAAC ATTCAAAGAG CTAACACAGT AAAGGTCATG
101  CAAGTTCTAG AATAGTGAAT CATGACAGAA CTCATTCATT TTATCCTTTA
151  TCTCC
```

TCU9/2(2)

```
  1  AAGTATGGGT AGCTAAATTT GCATTAAATT AAAAGTACAT ATAATGCAAC
 51  ACCACTCTAC ATCTGTATAC CTACGAATGT ATGTGTACTA CACACCCTTA
101  AAATGTTTTT CAAAGTCTTA ATATATTAGA ACATGTTTTC ATTTTTTCAT
151  GGGATGTTAA TACTATTCTA TGATTAAGAA AATACTAG
```

TCU10(2)

```
  1  AATACAGTTA TTCTAGCTTT TCATATTCAA TTTGAATGAT CAGAAAAGTA
 51  TATTAGTCAC ACAGAATTAA ATATTTTAGA TAGTAAGAAT C
```

TCU14(1)

```
  1  ATCCTTAGTA AGTGGATTTT GGGGAAAAAA GCACCTGGGC TTCTGGTTCT
 51  TTTTGATAAT ATATAAAATT ATTCATTATG AGGTTGCAGT TGTTTGCAAA
```

TCU14(2)

```
  1  GAAGTGAAAG TCAGCCCTTT AGCTATTATT TATTGCTTTA TTAGAGCAGA
 51  GGGAAGTGAC ACTCATTGCC TTCACAGAGC TCTGCAGAAA TATATGCACA
101  GAGTGGTCAA TGCCAACATC TGAGTAAGTC TTCCAAA
```

TGO20(2)

```
  1  CAGAACATTA GGATTTATTC CTTGATTAGT TCAAATGATT TCAACAGCTG
 51  AATTCCTTGA GATGTGTAAG GCAGGTTGGT CCTTTGGATG GACTGTAGAC
101  TGAAACTTCC TATAACTGTA GTGATATGTA CACAGCTACA TAGCAAAGTG
151  CTTCATTATG AAAATGAAGA A
```

TGO20(1)

```
  1  CAGTGTGAGA GTCTCATTTC TATGCACAGT GTTTCTCAGG AGGATGGAGC
 51  TAGTTAGCTG TCTGTTGTCT GTAGCCCAGC TTGATAATGG AACTATACAG
101  CGAAGAGACA ATCTCTGGCA AGTTTTTGTA GAA
```

TGU5(C)

```
  1  TTAGAGTAAA ATTCCAAATA AATGCTTTGC TCCAAAATTA CACTAACCAG
 51  GCTGGGTCTC TATCATACAT CTTCAATACC CTCAAACCTA GATTGTAAAG
101  TGAAAAAAGT GATTAGCNNT TCCATTTGTT CATTCTGTCA CTCACATTCT
151  TAGGCATTTT AAGGATGAGC AACCTTTGTT TCAGAAAGGG TAAGTAATTA
201  GCCCCCTGGA GGTTACATAG TTATAATTTA GTCTTCAGAA TCCGTTCGAA
251  GGGNNNNGTT ACTATTTTTA AGATAATTAG AACCCACCTT GTAGCAATAA
301  AAGTTTTCTT GTCTTTG
```

TGU8(2)

```
  1 GCGAAAGACT AATCGAACCA TCTAGTAGCT GGTTCCCTCC GAAGTTTCCC
 51 TCAGGATA
```

TGU9/1(2)

```
  1 TTAATGTTTA AATACTACTT TTTTTTCAAG CTTGCCCTAG ATACCAACTG
 51 TTTATCTAAC ACACAATTCC AGTGTTGCCA AGCCTCATGC CAATTTGAAG
101 GGAACAGCCA AAACTTATGC ATTCATATAA AAAGAGTCTC TAGGCTCTTA
151 TATCTACATT ATAATTTTT
```

TGU9/2(2)

```
  1 GGAATAACAT TTTTTTATGA GGGAACCCTT TAAAATGGAT GCACACAGTG
 51 GCATTTTCTC CTAGGCTCAA AGCTGAGTAC ACTCCCGTAA TTTTAATAAT
101 ATTTTAGGCA AGTCCTATGA CAATTATACC AACAAGTTTC TTCAACCCCA
151 CCACCACCCC ACCATCTCTA TGC
```

TGU12(C)

```
  1 GGAGGAAGCT TTATTTGGGA AGAGTGCGGT TCNNTCGGCC CTGATCAGCT
 51 CTAGCCTGCC CACCCCATCT CAGCCAGGCG GCTTTACTTC TTCCTGAGCT
101 TCAGGTCTTT CTTCTTCCTG ATTTCCTTGG CCAGCTCCCC AATCAATCTC
151 CAGTACTCAT TGAACTTGAG CTCCGAGNCC TGATTCACAT CCAAGCTCTT
201 CATCTTCT
```

TGU13/1(C)

```
  1 GGATGTGGTA GTTGATCTTT AATGCCCATT CTAGGTCGGA AAAATCCATG
 51 ATCCTAACTT TTAAGAGAAG GTTGGTAACT CTACTTAGGA CTTTTTTTTG
101 TAAGAGGAAT AATGTAGCCT CACCCTTATC TTTCTGGAAA TGTTTAAACC
151 ACTGAAATAT GGAGATCAAA TCCAGCTTAC ACACTGGTAA CTCAAATACT
201 ATTTTTTTTT TAAACTATCT TTTCTAAACT AATCACCCCT CTTGTACATA
251 GAACTTTCTA TCTCAGTGCC AATTCTTAGA GGTTGATGCA AACAGCTCTC
301 CAGAGAGCCT GTGCTATTGT TC
```

TGU13/2(2)

```
  1 GGGGTGTACA TTTTATTGGA AACCTTAAAT ACTGTTCAGA AAGAATATAT
 51 CTTCAATCAA GGTCTTGCCG AGCCTACACA GAAAAATGAA GCTTTTTGGG
101 TTAGGGGCAA GGATATATAC AGTACAGAGG ACAAAGA
```

TTO16/2(C)

```
  1 ACATTCATTA AAGATGAACT TTCAGCATCT TCACTTGAAG ATCCATCAGA
 51 TGATTCTGAG AGGCAGGTCT CCCCCAAAAA TCCACCGCAT GTATTCTTTC
101 GTTTAGAATC TGAAAGCCTC TTTCTTTTCA GGCTTGATGA CTCTTCTAAG
151 GTATTTGTTA TGCCTCTCTT CTGGGTTTTT CGTTTTGCCT TATCAAGTAG
201 CTNAAATTCA AACACCATGG CAANAGAAAC TGCTTCTAT
```

TTO20/1(C)

```
  1 CCACCAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTCAC AGTGAGATAT
 51 TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT
101 CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTTAAACCTG
151 GAGTTGATTA TACCATCACT GTGTATGCTG TCACTGGCCG TGGAGACAGC
201 CCCGCAAGCA GCAAGCCAAT TTCCATTAAT TACCGAACAG AAATTGACAA
251 ACCATCCCAG ATGCAAGTGA CCGATGTTCA AGACAACTGT TTTAATAAAA
301 GATTTACATT CCAC
```

TTO20/2(2)

```
  1 TTGGTACCAC AGTCACAGAA CTGGGGGTCA TTTTCTAGAT GAAACAAACG
 51 GAACAAGTTC TCTTCCAACA AAGAAATGTA CTGTAGAAAT TAATTTCCTC
101 CATGAATTTT ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA
151 AG
```

TTU2/1(2)

```
  1 CTAGAACTTC CAAAGGCTGC TTGTCATAGA AGCCATTGCA TCTATAAAGC
 51 AACGGCTCCT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAAGTC
101 ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCAATGC TTCTCG
```

TTU 2/2(C)

```
  1 AACCAGTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT
 51 TGTTTTCTTA TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG
101 CATGTTTTTC TTCATAGATA CCCGTTTTGG GGTTTTTGCG TCGGAAGATG
151 AAGTGCAGTT TATAGTCCTC TCCACATTTA TCTG
```

TTU3(1)

```
  1 GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATTCGG
 51 GAGGACCTAT TGGTGCGGGG GCTTTGTATG ATTATGGGCG TTGATTAGTA
101 GTAGTTACTG GTTGAACATT GTTTGTTGGT GTATATATTG TAATTGAGAT
151 TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG
201 AAG
```

TTU 5/1(2)

```
  1 GACAAAAAAA AAAAAAACAGG TTTTAAAGCT AGAAATGAAA AGCTACTTAA
 51 GTATCTTAAA GGATAAGTTA CTTTATTATA CACTAGAAAC ATACACAATA
101 GCTGAAAACT TAAAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG
```

TTU5/2(2)

```
  1 GCATCCATTG TACATTGTTT GGTTTGAGGT TACCATGAGG CCTGTAAATA
 51 CTATCTTATA ATTTATTATT TCAACCTGAT AAAACTTAAC ACTATTTGCA
101 TAAACAAACA AACGAAAA
```

```
TTU9/1(1)
        1  TAAAATACTG GTTCTTTTAT TCTGCAATAT TTTTAAAAAT CACATTTTCA
       51  GCCAGGCGCA GTTTCCCACA CCTGTAATCC GGCACTTTGG GAGGCTGAGA
      101  TGGGTGGATC ACAAGGTAGG AGATCAAACA TCCTGGCCAA CATGGTGAAC
      151  CTGTTTACT

TTU9/2(2)
        1  CAAGTATGGG TAGCTAAATT TGCATTAAAT TAAAAGTACA TATAATGCAA
       51  CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCTT
      101  AAATGTTTCA AAGCTTAATA TATTAGAACA TGTTTTCATT TTCAGGGAG

TTU13(2)
        1  GGAAATACAC TAGCATGTGA GCACTGTATA TAAAGCTTGA GGTTAGGAGG
       51  TAAAATGAAA GAAATCATTT TTAACTCCTA AGATGT

TTU13(1)
        1  TGAATTAAAT GGACTCGTTG AAAGGACAAG GAGATCGGTA ATATCTCTCT
       51  AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT
      101  CTTCTTTT
```

or an analog thereof.

11. Vector containing a DNA according to claim 10.

12. Host cell containing a vector according to claim 11.

13. Method for isolating a gene inducable by treating chondrocytes with IL-1β containing the steps:

    (a) hybridizing a DNA according to claim 10 under stringent conditions against DNA or RNA containing said gene; and
    (b) isolating said gene.

14. A method according to claim 13 wherein said DNA or RNA has been isolated from chondrocytes, particularly human chondrocytes, that were treated with IL-1β.

15. Process for expressing a gene isolated according to claims 13 or 14 containing the steps:

    (a) cloning said gene into a suitable expression vector; and
    (b) expressing said gene in a suitable host cell.

16. Method for producing a protein containing the steps:

    (a) culturing a suitable host cell containing a vector which contains a DNA according to claim 10 or a gene produced by a method according to claim 13 or 14; and
    (b) isolating the expressed protein.

17. Diagnostic aid containing a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof.

18. Use of a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof for the diagnosis, prophylaxis or therapy of IL-1β mediated diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

19. Use of a gene isolated according to claim 13 to 14 for the production of a pharmaceutical.